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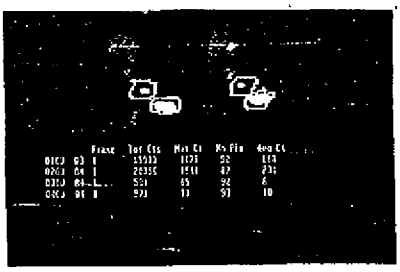

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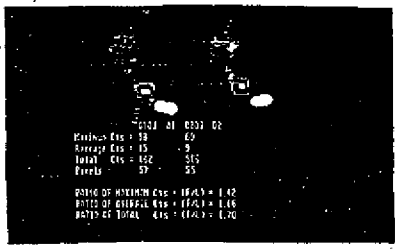

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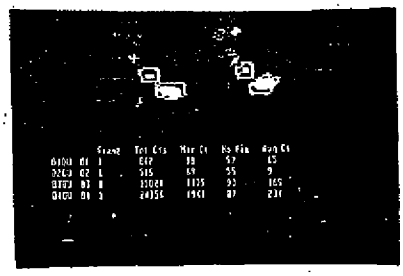

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(57) Abstract: This invention discloses the concept of incorporating a radioactive agent and various dyes to enhance lymphatic drainage, sentinel and lymph nodes. The use of a gamma emitting radionuclide such as Tc-99m allows the localization of the lymph node(s) that allows the surgeon to initially plan the surgical procedure. On the day of the study the radiopharmaceutical may need to be injected imaged and with the skin marked externally to assist the surgeon in locating the node during surgery. In order to facilitate the surgical probe a gamma detecting surgical probe can assist in providing the relative location of the node. This procedure has been found to be useful, however it has often been difficult utilizing this procedure to find all of the nodes. This invention would incorporate the use of a radioactive probe with a dye, the addition of the dye into the particles would allow the physician to more rapidly indentify lymphatic channels, sentinel and other node(s) and allow them to be excised which would dramatically reduce the surgical time for the patient.

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Radiopharmaceuticals and Methods for Imaging

Field of the Invention

The invention relates to medical diagnostic imaging and in particular imaging agents, kits and methods for medical diagnostic imaging.

Background of the Invention

Diagnostic imaging exploits agents that bind or localize to sites selectively within the body. Techniques of imaging include positron emission tomography, nuclear magnetic resonance imaging, scintigraphy, single photon emission computed tomography, perfusion contrast echocardiography, ultrafast X-ray computed tomography, and digital subtraction angiography. These techniques are used to diagnose many diseases, disorders and abnormal physical states, such as cancer, neurological abnormalities, inflammation, infection, and degenerative diseases.

Lymphoscintigraphy is a useful diagnostic imaging technique. The recognition of the importance of lymphoscintigraphy, for identification of the sentinel lymph node(s) in melanoma and breast cancer plays a significant role in the clinical management of patients. The widespread clinical acceptance of this technique and the lack of an agreement on which radiopharmaceutical agent has the most ideal properties has resulted in a wide variety of agents being used clinically with many other agents being investigation or developed.

Lymphoscintigraphy has resurged as a valuable technique for the identification of lymphatic drainage pathways and the location of the sentinel node(s). The renewed interest is largely due to a multidisciplinary approach validating the importance of the pathology of the sentinel node. A pathological finding in the node is an important factor in a patient's prognosis, management and clinical care. The exploration and use of the technique has a 30 year history over which time numerous radioactive tracers, colored dyes, and combined approaches have been investigated to identify lymphatic drainage and the sentinel node(s).

Animal studies have demonstrated that the particle size of a radiopharmaceutical agent is a critical factor in determining the migration rate from the injection site and the rate of uptake in lymph nodes. The particles should be larger than 0.005 nm in size, as smaller particles may penetrate or leak into the capillary membranes and therefore become unavailable to migrate through the lymphatic channel (1). Particles between 0.005 nm and 5 nm in size are able to migrate from an intradermal injection site into the lymphatic vessels. The particles move

through the lymphatic system by rhythmical contractions and relaxations of the smooth muscle cells in the capillary walls. Muscular activity and respiratory movement increase the lymph pressure, thus increasing lymphatic flow. Anesthesia may decrease lymphatic flow, however the magnitude of depression can vary considerably depending upon the anesthetic used (2). Following migration from the interstitial space and into the lymphatic vessel the particles are transported to the lymph nodes where they can be retained by mechanical trapping or phagocytosis. The optimal particle size identified from the animal studies for lymphatic drainage has been estimated to be about 5 nm (2). Larger particles approximately 500 nm in size demonstrate a much slower rate of migration from the injection site and significantly lower accumulation in the lymph nodes. Larger particles, greater than a few hundred nanometers in size are retained primarily at the injection site in the interstitial space. The size dependence for particle absorption and movement has been verified in both animal and human studies. The number of injected colloid particles has also been reported to influence the rate of out flow from the injection site and phagocytosis within the lymph nodes.

Radiopharmaceutical Agents

There have been many radiopharmaceuticals that have been evaluated and used for lymphoscintigraphy studies. Au-198 colloid was the first agent which was widely used but was rapidly replaced by other radionuclides and radiopharmaceuticals. The agents that are commonly used are Tc-99m antimony trisulfide colloid, Tc-99m nanocolloid and Tc-99m sulfur colloid and these agents are available in different parts of the world. In Europe the predominant agent used is Tc-99m nanocolloid whereas in North America, Tc-99m sulfur colloid is the primary agent of choice.

Colloidal Gold Au-198

One of the first agents widely used for lymphoscintigraphy was colloidal Au-198. Colloidal gold has a relatively uniform particle size of 3 to 5 nm, which is optimal (3-5) and was used clinically for many years to study the lymphatic system. Au-198 has a 2.7 day half life, emits beta particles matter and a 412 KeV gamma photon. Although it has favorable particle size properties, it is no longer widely used as it delivers a high radiation dose at the site of injection and has decreased spatial resolution due to its 412 KeV gamma photon. In addition, tissue necrosis

at the injection site was sometimes observed due to the large absorbed dose from electrons emitted from the beta decay of Au-198 (6,7).

Tc-99m Antimony Trisulfide Colloid

The first particulate Tc-99m agent to be used for lymphoscintigraphy was Tc-99m antimony trisulfide colloid. The colloid has a particle size range of 3 to 30 nm and has been used clinically in the last decade in various locations (6). Tc-99m labeling of antimony trisulfide colloid has been proposed to occur on the surface of the particles with the final particle size determined by the size of the antimony colloid used (8) (9). This agent was being developed when the clinical importance of lymphoscintigraphy studies was not widely recognized, therefore the radiopharmaceutical agent was never developed commercially worldwide.

Tc-99m Albumin Based Colloid Radiopharmaceuticals

Three types of albumin-based Tc-99m colloid radiopharmaceutical agents have been studied; nanocolloid, microaggregated albumin and macroaggregated albumin.

Tc-99m Nanocolloid is available as a kit containing human albumin nanocolloid particles and stannous chloride dihydrate. Approximately 95% of the colloidal albumin particles are smaller than 80 nm in size (10,11). Less than 4% of the particles are between 80-100 nm in size. There is about 1% of the particles that is larger than 100 nm (10,11). The preparation of Tc-99m Nanocolloid involves the addition of pertechnetate to a lyophilized vial of human albumin nanocolloid particles, stannous chloride, glucose, polyoxamer 238, sodium phosphate and sodiumphytate. It is critical to exclude oxygen from the vial during the addition of the pertechnetate as the oxygen will form a stannous technetium colloid and not allow the Tc-99m to bind to the albumin particles. Tc-99m Nanocolloid is being used routinely clinically in Europe.

Tc-99m microaggregated albumin and Tc-99m macroaggregated albumin have also been evaluated for lymphoscintigraphy studies. Tc-99m microaggregated albumin has a particle size distribution range of 0.2 to 2 μm in size with 90% less than 1 μm in size. Tc-99m macroaggregated albumin forms larger particles, ranging in size from 10 to 90 μm. Both of these agents have been shown to have slow migration from the injection site as would be expected due to their larger particle size. Tc-99m microaggregated albumin moves more rapidly than Tc-99m macroaggregated albumin and these agents have not been found to be very useful for

lymphoscintigraphy (12,13). However, there are some authors which report reasonably good studies utilizing Tc-99m microcolloid (14,15).

Tc-99m Sulfur Colloid

Tc-99m sulfur colloid has been evaluated as a potential radiopharmaceutical agent and numerous reports have demonstrated its usefulness for lymphoscintigraphic studies (9,16-22). Historically, there have been several methods of producing Tc-99m sulfur colloid particles by utilizing various starting materials and stabilizing agents. The routine preparation of Tc-99m sulfur colloid results in a preparation which has a very wide particle size distribution, ranging from <0.1 nm to greater than 5 μm (Table 1). The most common kit reagent uses sodium thiosulfate as the source of sulfur. The ingredients have been used in different amounts to develop kits which produce the appropriate particle size and having different degrees of stability (16,18,23) (17). Tc-99m sulfur colloid is formed by the reaction of thiosulfate under acidic conditions. Under these conditions there are two types of reactions which take place. One reaction involves the reaction of thiosulfate to form sulfur and bisulfite which also forms polythionates and subsequently high molecular weight sulfur and oxygen polymers. The reaction rates, the nature of the reactions, and the yields of the various products depends upon the thiosulfate concentration, acidity, and temperatures. The second type of reaction is an internal oxidation and reduction of thiosulfate in the presence of technetium which forms insoluble sulfides or stable sulfide complexes. Larson *et al.* (8)reported that upon heating there is rapid incorporation of Tc-99m into the sulfur colloid particles. The nucleation process of the reaction has been studied and it has been reported that the Tc colloid particles form more rapidly than the sulfur colloid. Thus, the sulfur colloid forms at least in part with Tc-colloid serving as its nucleus. In addition, some sulfur molecules form independently. The smaller particles generally contain relatively low amounts of sulfur and larger amounts of technetium (8,9).

The use of Tc-99m sulfur colloid for lymphoscintigraphy initially used the standard commercial formulation, however the success and reliability rate with this formulation was extremely variable. Recently, filtering the preparation prior to injection and/or modifying its method of production has provided a good radiopharmaceutical agent. Several reports have been published which state that filtering a standard sulfur colloid preparation through various sizes of membrane filters increases the quality of the preparation for lymphoscintigraphy (17,23). An unfiltered Tc-99m sulfur colloid kit was prepared and then filtered through a 0.1 μm membrane

filter, after filtration the average particle size distribution had a range of 10 nm with a small (< 0.1%) secondary population averaging 89-173 nm (23). Other studies have demonstrated that the use of a 0.22 μm membrane filter also gave a product that could image lymphatic drainage to identify the sentinel node(17,18).

An alternative to filtering the standard Tc-99m sulfur colloid preparation would be to alter the labeling procedure. Altering the preparation parameter has been reported to provide an agent which contains particles small enough to visualize the lymphatic drainage and particles large enough for prolonged retention within the lymph node to enhance the utility of the study. To achieve this Eshima et al (16) evaluated the particle size distribution and stability of the Tc-99m sulfur colloid kit utilizing different labeling conditions. The particle size distribution and the stability of the different Tc-99m sulfur colloid kit preparation parameters were evaluated over a 6 hour period utilizing polycarbonate filtration. The optimal labeling conditions required the addition of pertechnetate that had the longest ingrowth of Tc-99 pertechnetate, heating the reaction for 3 minutes, allowing the reaction to cool for 2 minutes and then neutralizing the reaction.

Tc-99m sulfur colloid has a variable particle size distribution pattern. All Tc-99m sulfur colloid preparations have a bimodal distribution pattern, regardless of the preparation procedure utilized (16). The use of a reduced heating protocol results in a dramatic increase in the percentage of particles smaller than 0.3 μm in size, regardless of the age of the generator elution while prolonged heating significantly decreases the percentage of small particles. In addition, this preparation does not appear to form a significant amount of particles smaller than 0.005 nm as there is no evidence of visualization or localization of activity outside of the lymphatic system. This preparation procedure demonstrates rapid movement of the particles and utilizing rapid dynamic images it has been able to map and imaging the lymphatic drainage system (24). In addition to rapid movement, the preparation has demonstrated prolonged retention within the nodes. Furthermore, the particle size distribution of the modified preparation of the kit formulation following filtration did not change over a six hour period (25).

Currently, several studies are underway to identify additional methods which would further reduce the average particle size formed during the preparation of Tc-99m sulfur colloid. Particle size studies utilizing Tc-99m sodium pertechnetate which has been obtained from generators which have up to 7 days of ingrowth of Tc-99 pertechnetate are being investigated. Also, studies in which an additive such as Re has been introduced into the kit demonstrated that there are more

nucleation sites for the particles to form. To optimize the percentage of small particles, a rhenium sulfide colloid kit formulation has been formulated and developed. TCK-17 is a kit formulation which utilizes rhenium sulfide colloid for the preparation of the agent. The addition of cold rhenium provides additional nucleation sites which allows for the formation of smaller size particles (26). In-vitro particle size distribution studies were conducted with the Tc-99m TCK-17 kit, these studies demonstrated a more homogeneous particle size distribution and no bimodal distribution pattern. In addition, there was a significantly larger percentage of particles smaller than 0.03 μm (63%). Preliminary animal studies demonstrated that there is a very rapid migration from the injection site; however additional studies need to be conducted to determine if the agent has significant retention in the lymph nodes (27).

Miscellaneous Lymphoscintigraphy Agents

Technetium-99m human serum albumin (Tc-99m HSA) has been evaluated for lymphoscintigraphy studies and, following an intradermal injection, has been used to image lymphatic flow. However, the agent is not particulate and there is minimal retention of the agent within the lymph nodes. In addition, delayed images may miss the sentinel node which makes it a suboptimal agent for use with the intraoperative probe (27,32). There are a number of other agents that have been used or are being developed for lymphoscintigraphy including Hg-197 sulfide colloid, Ga-67 citrate, and monoclonal antibodies labeled with In-111, I-131 and I-125 (2)

Although several of these agents have been utilized with various degrees of success the use of Tc-99m agents are more common due to its availability and favorable radionuclidic properties. Tc-99m gives comparatively low radiation exposures to patients and staff, has an optimal photon energy for scintillation camera imaging and surgical probes have been developed to localize the sentinel node during the surgical procedure. Several other Tc-99m based radiopharmaceutical agents have been evaluated as potential lymphoscintigraphy agents including hydroxyethyl starch (Tc-99m HES), dextran (Tc-99m DXT), and Tc-99m stannous phytate (1,33,34).

Problems have arisen utilizing a dye alone due to movement of the patient following imaging and positioning of the patient during surgery. The development of an intraoperative surgical probe has facilitated localization of the node but this procedure is not ideal. There is a

need for an imaging method to precisely identify the lymphatic drainage or nodes so that surgery to excise a node can be completed quickly and easily.

Summary of Invention

The invention relates to a double tracer technique to locate a node, preferably the sentinel node. Visible blue dyes such as isosulphan blue (28) (29), lymphazurin (30), or patent blue dye (PBD) (31) have been coinjected along with radiopharmaceutical agents for lymphoscintigraphy studies. In most cases, the addition of a blue dye serves as a useful adjunct to the radiopharmaceutical in the identification and surgical removal of the sentinel node. The invention relates to a radiopharmaceutical agent including a probe (preferably radioactive) and a dye and methods of using the dye in diagnostic imaging, preferably lymphoscintigraphy for diagnosis of cancer. The agents are also useful to identify drug delivery sites and the could be used to incorporate a therapeutic agent such as a chemotherapeutic agent or other useful toxins which could be released at the site.

The invention relates to a method of imaging a tissue in a mammal, comprising: a) administering to the mammal an effective amount of an agent including (i) a probe (ii) a dye, wherein the agent is capable of interacting with the tissue for detection; b) detecting the probe; and c) detecting the dye. In one embodiment, the method of claim 1, wherein the probe is radioactive. The probe is preferably connected to the dye. The tissue includes lymphatic tissue or a lymphatic node. The dye can be a coloured dye. The dye is preferably selected from the group consisting of a nonfluorescent dye, a fluorescent dye, an ultraviolet fluorescent dye, a visible fluorescent dye, an infared fluorescent dye, a chemiluminescent dye, a phosphorescent dye and a bioluminescent dye. The dye may be selected from the group consisting of:

Food dyes Flavoproteins Indocyanine Green (ICG) Fluorescein and disodium fluorescein Fluorescein isothiocyanate (FITC)	Methylene Blue a Porphyrin dye Bromosulfophthalein (BSP) Rose Bengal Isosulfan Blue	Evans Blue Tolonium Chloride Congo Red Hydroxy-pyrenetrisulfonate (HPT) a Pyrene-based dye	a Coumarin-based dye Luciferins Green Fluorescent Protein (GFP) an Acridinium-based dye Cibacron Blue; and an Inorganic Dye.

The radioactive probe can be a gamma emitting radionuclide. The radioactive probe preferably is a metal or a radioisotopic metal selected from the group consisting of Tc, Re, Mn, Fe, Co, Ni, Zn, Cd, Mo, W, Cu, Ag, Au, Ti, Hg, Cr and Rh, a halogen, Br, I, Cl, F, At. The imaging is preferably done with a technique selected from the group consisting of lymphoscintigraphy, scintigraphy, X-ray contrast, Barium particle imaging, positron emission tomography, nuclear magnetic resonance imaging, single photon emission computed tomography, perfusion contrast echocardiography, ultrafast X-ray computed tomography, digital subtraction angiography, spiral CT, gamma probe detectors, hand held gamma probe detectors, fMRI (fast Magnetic Resonance Imaging) and standard X-Ray equipment.

The probe includes a probe selected from the group consisting of hydroxyethyl starch, dextran, stannous phytate, sulfide colloid, sulfur colloid, citrate, a monoclonal antibody, a polyclonal antibody, human serum albumin antimony trisulfide colloid, nanocolloid, albumin, albumin-based colloid, nanocolloid albumin, microaggregated albumin and macroaggregated albumin. The probe can be selected from the group consisting of hydroxyethyl starch (Tc-99m HES), dextran (Tc-99m DXT), Tc-99m stannous phytate, lymphoscintigraphy, Hg-197 sulfide colloid, Ga-67 citrate, a monoclonal antibody labeled with In-111, I-131 or I-125, Tc-99m human serum albumin (Tc-99m HSA), Tc-99m antimony trisulfide colloid, Tc-99m nanocolloid, Tc-99m sulfur colloid, albumin-based Tc-99m colloid, nanocolloid, microaggregated albumin and macroaggregated albumin.

The agent can be administered by methods including intradermally, subcutaneously, bydirect lymphatic injection into a lymphatic channel, by intravenous injection or by intraarterial injection. The radioactive probe may be detected with a gamma camera. The dye may be detected with a uv light source.

Another aspect of the invention relates to a method of diagnosing cancer in a mammal, by administering to the mammal an effective amount of an agent including (i) a probe and (ii) a dye, wherein the agent is capable of migrating through a lymphatic channel into a lymphatic node for detection; detecting the probe and thereby detecting the location of the lymphatic node; detecting the dye and thereby detecting the location of the lymphatic node; removing the node; and determining the presence or absence of cancer in the node. The node preferably comprises a sentinel node. The cancer includes breast cancer or melanoma.

Another aspect of the invention relates to a method of imaging and assessing lymphatic drainage or a node, comprising: administering to the mammal an effective amount of an agent including (i) a probe and (ii) a dye, wherein the agent is capable of migrating through a lymphatic channel for detection; detecting the probe; and detecting the dye. The method may further include removing the node, preferably a sentinel node. The probe is preferably at least about 0.05nm in size and about 5nm in size. The dye can be any suitable dye disclosed in this application or known in the art, such as FITC. The probe can be any suitable probe disclosed in this application or known in the art and is preferably selected from the group consisting of a peptide, a polypeptide, a protein, an antibody, Tc-99m sulfur colloid and Tc-99m colloidal albumin. The antibody can be a polyclonal antibody or a monoclonal antibody or a fragment of either of the foregoing.

Another aspect of the invention includes a method of medical treatment of cancer in a mammal, by administering to the mammal an effective amount of an agent including (i) a probe which selectively interacts with a cancer cell and (ii) a dye; detecting the probe and thereby detecting the location of the cancer cell; detecting the dye and thereby detecting the location of the cancer cell; administering an anti-cancer agent or treatment proximate to the cancer cell. The probe can include an antibody. The cancer can include liver cancer, melanoma or breast cancer.

Another aspect of the invention relates to a method of producing an imaging agent, comprising connecting a probe with a dye.

Another aspect of the invention is a composition including a carrier and an agent including (i) a radioactive probe and (ii) a dye. In a variation, the composition includes a carrier and an agent including (i) a radioactive probe and (ii) a dye, wherein the agent is capable of migrating through a lymphatic channel. The composition preferably includes a pharmaceutical composition. The carrier may include albumin particles, inert microspheres, sulfur colloid

particles or Re-Sulfur colloidal paricles. The composition may include a probe and dye for coinjection, as disclosed in this application.

Another aspect of the invention relates to a kit for imaging, inlcuding an agent including (i) a radioactive probe and (ii) a dye. In a variation, the invention includes a kit for imaging, including an agent including (i) a radioactive probe and (ii) a dye, wherein the agent is capable of migrating through a lymphatic channel. Another aspect of the invention relates to a kit for producing an imaging agent, including (i) a radioactive probe compound and (ii) a dye compound. The kit may include a probe and dye for coinjection, as disclosed in this application.

The invention also includes a method of detecting the presence or assessing of the severity of a disease, disorder or abnormal physical state in a mammal comprising: (a) administering an effective amount of a composition of the invention; and (b) detecting the presence or assessing the severity of the disease, disorder or abnormal physical state. The probe can be radioactive.

The invention also includes a method of imaging a tissue in a mammal, by: administering to the mammal an effective amount of (i) a probe (ii) a dye, other than a blue dye, wherein the agent is capable of interacting with the tissue for detection; detecting the probe; and detecting the dye. The probe can be radioactive. The dye is preferably selected from the group consisting of a nonfluorescent dye, a fluorescent dye, an ultraviolet fluorescent dye, a visible fluorescent dye, an infared fluorescent dye, a chemiluminescent dye, a phosphorescent dye and a bioluminescent dye.

Brief Description of the Drawings

Preferred embodiments of the invention will be described in relation to the drawings in which:

Figure 1(a) –(c) Node activity as measured by γ camera.

Figure 2(a)-(d) Node activity as measured by γcamera.

Figure 3 γ camera image and corresponding image taken under UV light.

Figure 4 (a) –(c) Node activity as measured by γ camera.

Figure 5(a) Activity of sulfur colloid fractions; (b) Activity of fractions of Tc99m sulfur colloid.

Figure 6(a) Activity of fractions of Tc99m sulfur colloid; (b) Fluorescence of FITC-Tc99m sulfur colloid vs. fraction; (c) Fluorescence vs. Fraction # for 1mg fluorescein; (d) Amount of activity per fraction of 99mTc-fluorescein-SC; (e) Amount of fluorescence per fraction of 99mTc-fluorescein-SC; (f) Node activity as measured by γ camera.

Detailed Description of the Invention

The invention relates to a radiopharmaceutical agent including a probe and a dye and methods of using the dye in diagnostic imaging, preferably lymphoscintigraphy for diagnosis of cancer. The probe is preferably radioactive. The agents are also useful to identify drug delivery sites and the could be used to incorporate a therapeutic agent such as a chemotherapeutic agent or other useful toxins which could be released at the site.

The probe and dye are preferably connected. The invention also includes methods of coinjecting probe and dye for imaging. If we coinject dyes, such as the fluorescent dyes it allows one to identify lymphatic flow and lead to the location of the sentinel node. In addition by co injection the dye may become trapped along with the radiolabeled probe which is useful in diagnostic imaging.

In addition to the blue dye there are numerous other dyes that can be incorporated into lymphoscintigraphy studies to identify lymphatic drainage, the sentinel node(s), or other nodes. These dyes may be incorporated either as an adjuvant in solution or incorporated into a particle for prolonged retention within the lymph node. Dyes that are encompassed in this patent application include nonflourescent, fluorescent (ultraviolet, visible and infrared), chemiluminescent, phosphorescent and bioluminescent dyes. Possible dyes that can be used include:

1. Food dyes 2. Flavoproteins 3. Indocyanine Green (ICG) 4. Fluorescein and disodium fluorescein 5. Fluorescein isothiocyanate (FITC)	6. Methylene Blue 7. Porphyrin dyes i.e., Pd-uroporphyrin 8. Bromosulfophthalein (BSP) 9. Rose Bengal 10. Isosulfan Blue	11. Evans Blue 12. Tolonium Chloride 13. Congo Red 14. Hydroxy-pyrenetrisulfonate (HPT) 15. Pyrene-based dyes i.e., 1,3-dihydroxy 6,8-pyrenedisodiumsulfonate	16. Coumarin-based dyes, i.e., 3-(carboxymethylester)-7-julolidinocoumarin 17. Luciferins 18. Green Fluorescent Protein (GFP) 19. Acridinium-based dyes 20. Cibacron Blue 21. Inorganic Dyes Such As Re(I) metal ligand complexes

This patent application demonstrates that FITC can be incorporated into Tc-99m sulfur colloid or albumin particles and be administered intradermally or subcutaneously. This combination approach allows the detection of the lymphatic drainage pathway and sentinel node(s) as well as other nodes easily by gamma camera scintigraphy. These nodes can be marked on the skin surface to more easily locate the node during the surgical procedure.

We have incorporated FITC into Tc-99m sulfur colloid and Tc-99m colloidal albumin particles. The methodology for incorporation of FITC are listed below.

1) Add Tc-99m in 3ml saline to sulfur colloid kit vial
Add syringe A to vial
Boil vial for 1.5 min
Add 15mg FITC/600ul DMF to vial
Boil vial for 1.5 mins
Cool vial for 2 mins at room temperature
Filter through a 0.22 micron filter and use or
Add 0.7 ml of this to 0.3ml FITC/DMF (25mg/ml) and use

2) Add Tc-99m to Microlite kit vial and Vortex briefly
Add 250ul of NaHCO3 (1M) pH 8.5
Vortex for 10 seconds
Add 500ul of 10mg/ml FITC in DMF

Vortex for 10 seconds

Incubate for 1hr at 4°C and use or

Filter through a 0.22 micron filter and use

The methodology given above may be readily adapted or modified by one skilled in the art.

Following the preparation of the radiolabeled and UV agent the material was injected intradermally or subcutaneously into a rat. Dynamic images were obtained and movement of the radioactivity was monitored with the gamma camera. At 1.5 hours post injection, the animals were sacrificed and an incision was made and a UV light source was used to readily localize the node by emitting a yellow to green fluorescent color.

A modified labeling procedure was also used to incorporate fluorescein into the Microlite particles and similar results were obtained; gamma camera visualization of the flow of particles and after an incision was made the UV light was able to rapidly localize the sentinel node.

There are other methods that can be utilized to incorporate or attach dyes to radioactive particles which will be apparent to those skilled in the art.

Metal

Suitable metals include the transition metals, lanthanide metals, halogens and actinide metals. Complex-forming metals useful in preparing agents for radiotherapy or imaging are preferably the metals (or radioisotopes of the metals) Tc, Re, Mn, Fe, Co, Ni, Zn, Cd, Mo, W, Cu, Ag, Au, Ti, Hg, Cr, Rh., At, Gd, Ga, Ho, In, Lu, Sm, Yb and Y.

Non-Metal Agents may also be useful and can include, F, CL, Br and At. These can be attached utilizing standard labeling procedures.

The probe does not have to be radioactive for all applications of the invention. For example, in MRI, fMRI, X-Ray, CT and Ultrasound it would not have to be radioactive.The halide salt, in particular chloride salt, or oxide of these complex-forming metals are forms capable of complexing with a desired ligand and are suitable for the present invention. Radionuclide labeled imaging agents employ complex-forming metal isotopes that include ß-emitters such as rhenium-186 and -188; and γ-emitters such as technetium-99m. The complex-forming metal most preferred for radiodiagnostic imaging is technetium-99m due to its advantageous half life of 6

hours and inexpensive preparation from a molybdenum-99 generator. Technetium and rhenium labeling is accomplished by procedures established in the art. Either complex-forming metal may be introduced to the ligand in aqueous solution in oxo, dioxo or nitrido form, for example pertechnetate ($^{99m}TcO_4^-$) or perrhenate, with a suitable reducing agent such as stannous chloride. Alternatively, radiodiagnostic agents may be formed by a transchelation reaction which entails use of the complex-forming metal in the form of a weak metal complex such as technetium-gluconate, heptagluconate, tartrate or citrate to give a desired labeled ligand. Transchelation reactions are typically heated to facilitate conversion of technetium from the weak complex to a complex with the ligand, for example in a boiling hot water bath.

There are two general methodologies which can be utilized to make a combined probe-dye conjugate. The first methodology requires the covalent attachment of the dye directly to the probe. The probe can then subsequently be radiolabeled utilizing standard labeling methodologies. Alternatively, the probe may be radiolabeled followed by the addition of the dye via a covalent attachment to the radiolabeled probe. The second methodology involves incorporation into the probe utilizing a number of different techniques. Some of which include; modifications of the labeling procedure for sulfur colloid particles so as to incorporate the dye during the heating step while the particles are being formed during the radiolabeling procedure. An alternative method would be to allow the dye particles to incubate with the probe and therefore be allowed to bind to the particles through electrostatically or other non-covalent properties. The probe can then be radiolabeled prior to the binding or after the radiolabeling of the probe.

Pharmaceutical Compositions

The invention also includes compositions, preferably pharmaceutical compositions for radiotherapy or imaging, including an agent prepared according to a method of the invention. Pharmaceutical compositions may be formulated according to known techniques.

The invention includes a method of detecting the presence or assessing of the severity of a disease, disorder or abnormal physical state in a mammal comprising: (a) administering an agent or composition of the invention and (b) detecting the presence or assessing the severity of the disease, disorder or abnormal physical state. The presence or severity of the disease, disorder or abnormal physical state is preferably detected or assessed with lymphoscintigraphy, or

a technique selected from the group consisting of positron emission tomography (PET), magnetic resonance imaging (MRI) scintigraphy, single photon emission computed tomography, perfusion contrast echocardiography, ultrafast X-ray computed tomography, digital subtraction angiography, spiral CT, hand held gamma probe detectors, fMRI (fast Magnetic Resonance Imaging) and standard X-Ray equipment. Suitable methods and materials for imaging are described in: Handbook of Nuclear Medicine second ed., 1993, Mosby Press, Frederic I. Datz; Fundamentals of Nuclear Pharmacy third ed., 1992 Springer-Verlag, Gopal B. Saha; Principles and practice of Nuclear Medicine second ed., 1995, Mosby press, Paul J. Early and D. Bruce Sodee; which are incorporated by reference in their entirety.

The invention also includes a method of radiotherapy of a disease, disorder or abnormal physical state in a mammal including administering an agent or composition of the invention. Methods of performing radiotherapy are described in, for example, Principles and Practice of Nuclear Medicine, 2nd Ed., P.J. Early and D.B. Sodee, Chapter 32, which is incorporated by reference in its entirety.

The particles could be used therapeutically by incorporating an alpha or beta radionuclide into the probe. After visual localization of the node and demonstrating that the probe is being retained in the lymph node the therapeutic radionuclide could deliver a large radiation dose to destroy the tumor cells. This would be especially useful for those probes which may not be able to be surgically removed because of their location or the morbidity which might result in removal of the node.

The pharmaceutical compositions are used to treat diseases and provide images in diseases, disorders or abnormal physical states including cancer. Other diseases, disorders and abnormal physical states will be apparent to those skilled in the art and/or on review of this application or references cited in this application.

Pharmaceutical compositions used for imaging or to treat patients having diseases, disorders or abnormal physical states preferably include an agent of the invention and an acceptable vehicle or excipient (Remington's Pharmaceutical Sciences 18th ed, (1990, Mack Publishing Company) and subsequent editions). Vehicles include saline and D5W (5% dextrose and water) or other acceptable injection vehicles. Excipients include additives such as a buffer, solubilizer, suspending agent, emulsifying agent, viscosity controlling agent, lactose

filler, antioxidant, preservative or disintegrants. The compositions may further include a reducing agent, a bulking agent or a pH stabilising agent. There are preferred excipients for stabilizing peptides for parenteral and other administration. The excipients preferably include serum albumin, glutamic or aspartic acid, phospholipids and fatty acids. Intradermal, subcutaneous, direct lymphatic injection or parenteral (injectable) administration can be utilized. The methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients are known in the art.

The pharmaceutical compositions can be administered to humans or animals (preferably mammals). Dosages to be administered depend on individual patient condition, indication of the drug, physical and chemical stability of the drug, toxicity, the desired effect and on the chosen route of administration (Robert Rakel, ed., Conn's Current Therapy (1995, W.B. Saunders Company, USA)). Preferably the amount of complex-forming metal labeled agent administered to the mammal is approximately 100 to 1000 microcuries of activity for an intradermal injection injected around the primary site for melanoma studies, in the case of identification of lymph nodes in breast cancer 1 to 5 mci may be injected around the primary site. about 0.01 mcg/kg/minute to 1,000 mcg/kg/minute and more preferably about 0.01 to 50 mcg/kg/minutes.

Kits

The invention also includes kits for imaging, and preferably lymphoscintigraphy. Since the radioisotopic metals often have a very short half life, it is advantageous to omit them from the kit.

The metal is preferably one or more of the metals and radioisotopic metal forms of Tc, Re, Mn, Fe, Co, Ni, Zn, Cd, Mo, W, Cu, Ag, Au, Tl, Hg, Cr and Rh.

Preferred embodiments of the invention are described below in examples which are not intended to in any way limit the scope of the invention.

Example 1

We demonstrated that the FITC-TKPR co-injected with Tc-99m sulfur colloid could be detected in the sentinel node of the rat at 30 minutes, 1 hour, 1.5 hours and 2 hours post injection. The accompanying gamma camera images are in Figures 1(a)-(c). The FITC-TKPR

Tc-99m sulfur colloid was generated using the protocol outlined below and 100 µl was injected into each rat hindpad.

Add Tc-99m in 3ml saline to sulfur colloid kit vial
Add syringe A to vial
Boil vial for 3 minutes
Cool vial for 2 minutes at room temperature
Add syringe B
Filter Tc-99m sulfur colloid through 0.22-micron filter
Add 2mg FITC-TKPR/250ul to vial and use

Example 2

We demonstrated that the FITC-conjugated Tc-99m sulfur colloid could be detected in the sentinel node of the rat at 30 minutes, 1 hour, 1.5 hours and 2 hours post injection. This data supports the accompanying gamma camera images that are located in Figures 2(a)-2(d) and Figure 3. The FITC Tc-99m sulfur colloid was generated using the protocol outlined below and 100 µl was injected into each rat hindpad.

Add Tc-99m in 3ml saline to sulfur colloid kit vial
Add syringe A to vial
Boil vial for 1.5 min
Add 15mg FITC/600ul DMF to vial
Boil vial for 1.5 minutes
Cool vial for 2 minutes at room temperature
Filter through a 0.22 micron filter and use or
Add 0.7 ml of this to 0.3ml FITC/DMF (25mg/ml) and use

Example 3

We demonstrated that the FITC-conjugated Tc-99m Microlyte could be detected in the sentinel node of the rat at 30 minutes, 1 hour, 1.5 hours and 2 hours post injection. This data support the accompanying gamma camera images in Figures 4(a)-(c). The FITC-conjugated Tc-99m Microlyte was generated using the protocol outlined below and 100 µl was injected into each rat hindpad.

Add Tc-99m to Microlyte kit vial
Vortex briefly
Add 250ul of NaHCO3 (1M) pH 8.5
Vortex for 10 seconds
Add 500ul of 10mg/ml FITC in DMF
Vortex for 10 seconds
Incubate for 1hr at 4°C and use or
Filter through a 0.22 micron filter and use

Example 4

We demonstrated that Tc-99m sulfur colloid particles can be separated using a Pharmacia PD-10 column (G-25 column). A sample of Tc-99m sulfur colloid (2.5 ml) was loaded upon the column and then eluted off in 1 ml fractions. This data is presented in graph format in Figures 5(a) and (b). This data show that the Tc-99m sulfur colloid particles can be separated using a PD-10 column. The Tc-99m sulfur colloid was prepared using the following protocol.

Add Tc-99m in 3ml saline to sulfur colloid kit vial
Add syringe A to vial
Boil vial for 3 minutes
Cool vial for 2 minutes at room temperature
Add syringe B
Filter Tc-99m sulfur colloid through 0.22-micron filter and use

Example 5

We determined whether fluorescein could be incorporated into Tc-99m sulfur colloid particles during their formation (Figure 6(a),(b)). The fluorescein Tc-99m sulfur colloid was generated using the protocol outlined below. This data characterizes the elution pattern of the Tc-99m sulfur colloid off the PD-10 column. This data characterizes the elution pattern of the fluorescein off the PD-10 column. The results from this experiment showed that fluorescein was not incorporated into the Tc-99m sulfur colloid particles using the labeling protocol outlined below. Imaging was achieved by coinjecting the compounds.

Add Tc-99m in 3ml saline to the sulfur colloid kit vial
Add syringe A to vial
Boil vial for 2 minutes
Add 100ul of 50mg/0.1ml fluorescein
Boil vial for 1 minute
Cool vial for 2 minutes at room temperature
Add syringe B to vial
Filter though a 0.22 micron filter and use

We determined the elution pattern of fluorescein on a Pharmacia PD-10 column (G-25 column) as shown by Figure 6(c), .

Example 6

We determined whether fluorescein could be incorporated into Tc-99m sulfur colloid particles during their formation (Figures 6(d)-(f)). The fluorescein Tc-99m sulfur colloid was generated using the protocol outlined below. This data characterizes the elution pattern of the Tc-99m sulfur colloid off the PD-10 column. The data in the chart on page 50 and 51 of lab The fluorescein Tc-99m sulfur colloid was generated using the protocol outlined below and 100 µl was injected into each rat hindpad.

The results from this experiment showed that fluorescein was not incorporated into the Tc-99m sulfur colloid particles using the labeling protocol outlined below, however, the addition of fluorescein to the Tc-99m sulfur colloid kit did not interfere with the generation of functional particles.

Add Tc-99m in 3ml saline to the sulfur colloid kit vial
Add syringe A to vial
Boil vial for 1.5 minutes

Add 200ul of 50mg/0.1ml fluorescein
Boil vial for 1.5 minutes
Cool vial for 2 minutes at room temperature
Add syringe B to vial
Filter though a 0.22 micron filter and use

The present invention has been described in detail and with particular reference to the preferred embodiments; however, it will be understood by one having ordinary skill in the art that changes can be made thereto without departing from the spirit and scope of the invention.

All publications, patents and patent applications are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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We Claim

1. A method of imaging a tissue in a mammal, comprising:

a) administering to the mammal an effective amount of an agent including (i) a probe (ii) a dye, wherein the agent is capable of interacting with the tissue for detection;

b) detecting the probe; and

c) detecting the dye.

2. The method of claim 1, wherein the probe is radioactive.

3. The method of claim 1, wherein the probe is connected to the dye..

4. The method of claim 3, wherein the tissue comprises lymphatic tissue or a lymphatic node.

5. The method of any of claims 1 to 4, wherein the dye comprises a coloured dye.

6. The method of any of claims 1 to 5, wherein the dye is selected from the group consisting of a nonfluorescent dye, a fluorescent dye, an ultraviolet fluorescent dye, a visible fluorescent dye, an infared fluorescent dye, a chemiluminescent dye, a phosphorescent dye and a bioluminescent dye.

7. The method of claim 6, wherein the dye is selected from the group consisting of:

1. Food dyes 2. Flavoproteins 3. Indocyanine Green (ICG) 4. Fluorescein and disodium fluorescein 5. Fluorescein isothiocyanate (FITC)	6. Methylene Blue 7. a Porphyrin dye 8. Bromosulfophthalein (BSP) 9. Rose Bengal 10. Isosulfan Blue	11. Evans Blue 12. Tolonium Chloride 13. Congo Red 14. Hydroxy-pyrenetrisulfonate (HPT) 15. a Pyrene-based dye	16. a Coumarin-based dye 17. Luciferins 18. Green Fluorescent Protein (GFP) 19. an Acridinium-based dye 20. Cibacron Blue; and 21. an Inorganic Dye.

8. The method of any of claims 1 to 7, wherein the radioactive probe comprises a gamma emitting radionuclide.

9. The method of claim 8, wherein the radioactive probe comprises a metal or a radioisotopic metal selected from the group consisting of Tc, Re, Mn, Fe, Co, Ni, Zn, Cd, Mo, W, Cu, Ag, Au, Ti, Hg, Cr and Rh, a halogen, Br, I, Cl, F, At.

10. The method of any of claims 1 to 9, wherein the imaging is done with a technique selected from the group consisting of lymphoscintigraphy, scintigraphy, X-ray contrast, Barium particle imaging, positron emission tomography, nuclear magnetic resonance imaging, single photon emission computed tomography, perfusion contrast echocardiography, ultrafast X-ray computed tomography, digital subtraction angiography, spiral CT, gamma probe detectors, hand held gamma probe detectors, fMRI (fast Magnetic Resonance Imaging) and standard X-Ray equipment.

11. The method of any of claims 1 to 10, wherein the probe is selected from the group consisting of hydroxyethyl starch, dextran, stannous phytate, sulfide colloid, sulfur colloid, citrate, a monoclonal antibody, a polyclonal antibody, human serum albumin antimony trisulfide colloid, nanocolloid, albumin, albumin-based colloid, nanocolloid albumin, microaggregated albumin and macroaggregated albumin.

12. The method of claim 11, wherein the probe is selected from the group consisting of hydroxyethyl starch (Tc-99m HES), dextran (Tc-99m DXT), Tc-99m stannous phytate,

lymphoscintigraphy, Hg-197 sulfide colloid, Ga-67 citrate, a monoclonal antibody labeled with In-111, I-131 or I-125, Tc-99m human serum albumin (Tc-99m HSA), Tc-99m antimony trisulfide colloid, Tc-99m nanocolloid, Tc-99m sulfur colloid, albumin-based Tc-99m colloid, nanocolloid, microaggregated albumin and macroaggregated albumin.

13. The method of any of claims 1 to 12, wherein the agent is administered intradermally, subcutaneously, bydirect lymphatic injection into a lymphatic channel, by intravenous injection or by intraarterial injection.

14. The method of claim 1, wherein the probe is radioactive and is detected with a gamma camera.

15. The method of claim 1, wherein the dye is detected with a uv light source.

16. A method of diagnosing cancer in a mammal, comprising administering to the mammal an effective amount of an agent including (i) a probe and (ii) a dye, wherein the agent is capable of migrating through a lymphatic channel into a lymphatic node for detection;

a) detecting the probe and thereby detecting the location of the lymphatic node;
b) detecting the dye and thereby detecting the location of the lymphatic node;
c) removing the node; and
d) determining the presence or absence of cancer in the node.

17. The method of claim 16, wherein the node comprises a sentinel node.

18. The method of claim 16 or 17, wherein the cancer comprises breast cancer or melanoma.

19. A method of imaging and assessing lymphatic drainage or a node, comprising: administering to the mammal an effective amount of an agent including (i) a probe and (ii) a dye, wherein the agent is capable of migrating through a lymphatic channel for detection;

a) detecting the probe; and

b) detecting the dye.

20. The method of claim 19, further comprising removing the node.

21. The method of claim 20, wherein the node comprises a sentinel node.

22. The method of any of claims 16 to 21, wherein the probe is at least about 0.005nm in size.

23. The method of claim 22, wherein the probe is about 5nm in size.

24. The method of any of claims 16 to 23, wherein the dye comprises FITC.

25. The method of any of claims 16 to 24, wherein the probe is selected from the group consisting of a peptide, a polypeptide, a protein, an antibody, Tc-99m sulfur colloid and Tc-99m colloidal albumin.

26. The method of claim 25, wherein the antibody comprises a polyclonal antibody or a monoclonal antibody or a fragment of either of the foregoing.

27. A method of medical treatment of cancer in a mammal, comprising administering to the mammal an effective amount of an agent including (i) a probe which selectively interacts with a cancer cell and (ii) a dye;

a) detecting the probe and thereby detecting the location of the cancer cell;

b) detecting the dye and thereby detecting the location of the cancer cell;

c) administering an anti-cancer agent or treatment proximate to the cancer cell.

28. The method of claim 27, wherein the probe comprises an antibody.

29. The method of claim 27, wherein the cancer comprises liver cancer, melanoma or breast cancer or any other metastatic cancers.

30. A method of producing an imaging agent, comprising connecting a probe with a dye.

31. A composition comprising a carrier and an agent including (i) a radioactive probe and (ii) a dye.

32. A composition comprising a carrier and an agent including (i) a radioactive probe and (ii) a dye, wherein the agent is capable of migrating through a lymphatic channel.

33. The composition of claim 31 or 32, wherein the composition comprises a pharmaceutical composition.

34. The composition of any of claims 30 to 33, wherein the carrier comprises albumin particles, inert microspheres, sulfur colloid particles or Re-Sulfur colloidal paricles.

35. A kit for imaging, comprising an agent including (i) a radioactive probe and (ii) a dye.

36. A kit for imaging, comprising an agent including (i) a radioactive probe and (ii) a dye, wherein the agent is capable of migrating through a lymphatic channel.

37. A kit for producing an imaging agent, comprising (i) a radioactive probe compound and (ii) a dye compound.

38. A method of detecting the presence or assessing of the severity of a disease, disorder or abnormal physical state in a mammal comprising: (a) administering an effective amount of the composition of any claims 31 to 34; and (b) detecting the presence or assessing the severity of the disease, disorder or abnormal physical state.

39. The method of any of claims 15 to 30, wherein the probe is radioactive.

40. A method of imaging a tissue in a mammal, comprising:

a) administering to the mammal an effective amount of (i) a probe (ii) a dye, other than a blue dye, wherein the agent is capable of interacting with the tissue for detection;

b) detecting the probe; and

c) detecting the dye.

41. The method of claim 40, wherein the probe is radioactive.

42. The method of claim 40 or 41, wherein the dye is selected from the group consisting of a nonfluorescent dye, a fluorescent dye, an ultraviolet fluorescent dye, a visible fluorescent dye, an infared fluorescent dye, a chemiluminescent dye, a phosphorescent dye and a bioluminescent dye.

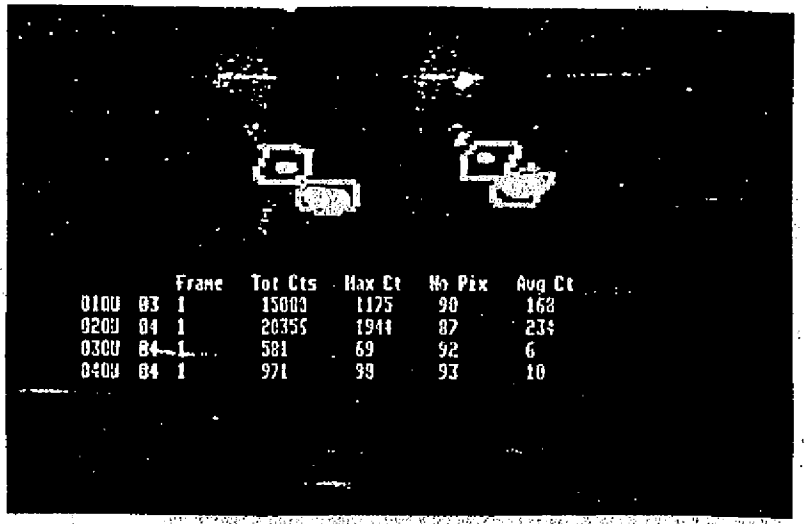

Figure 1a

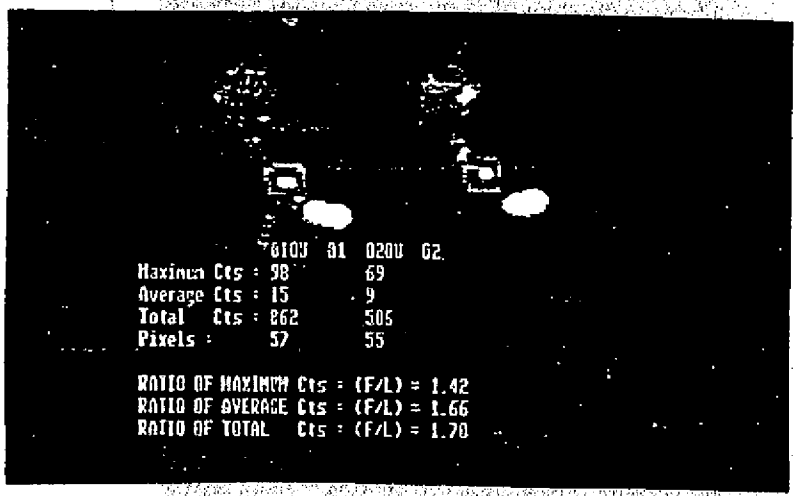

Figure 1b

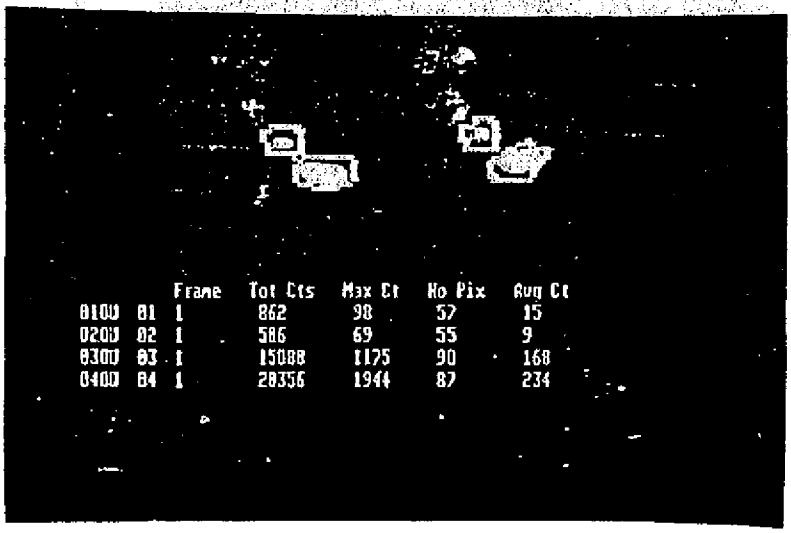

Figure 1c

Figure 2a

Figure 2b

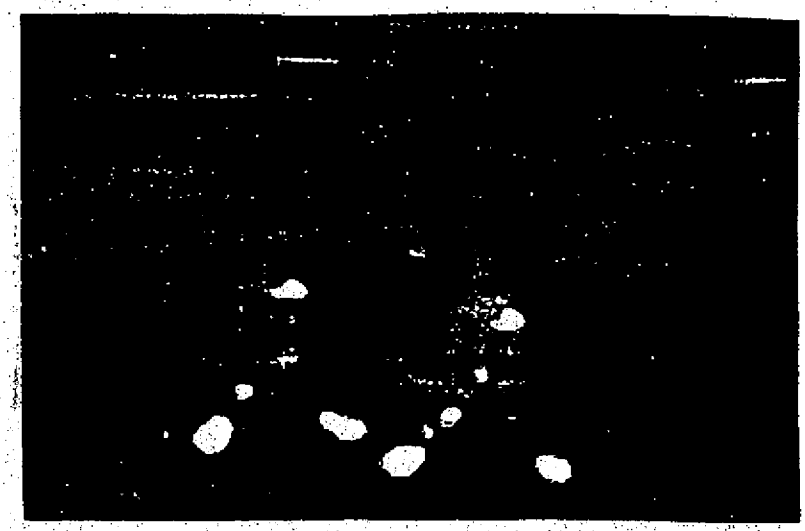

Figure 2c

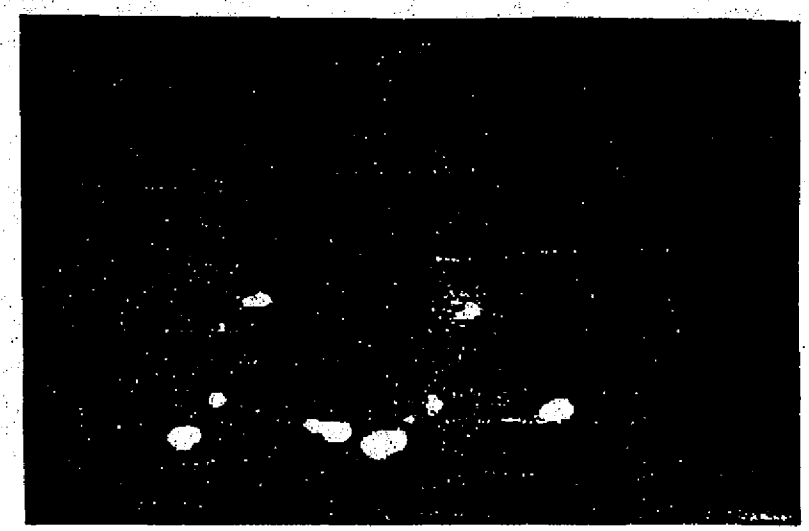

Figure 2d

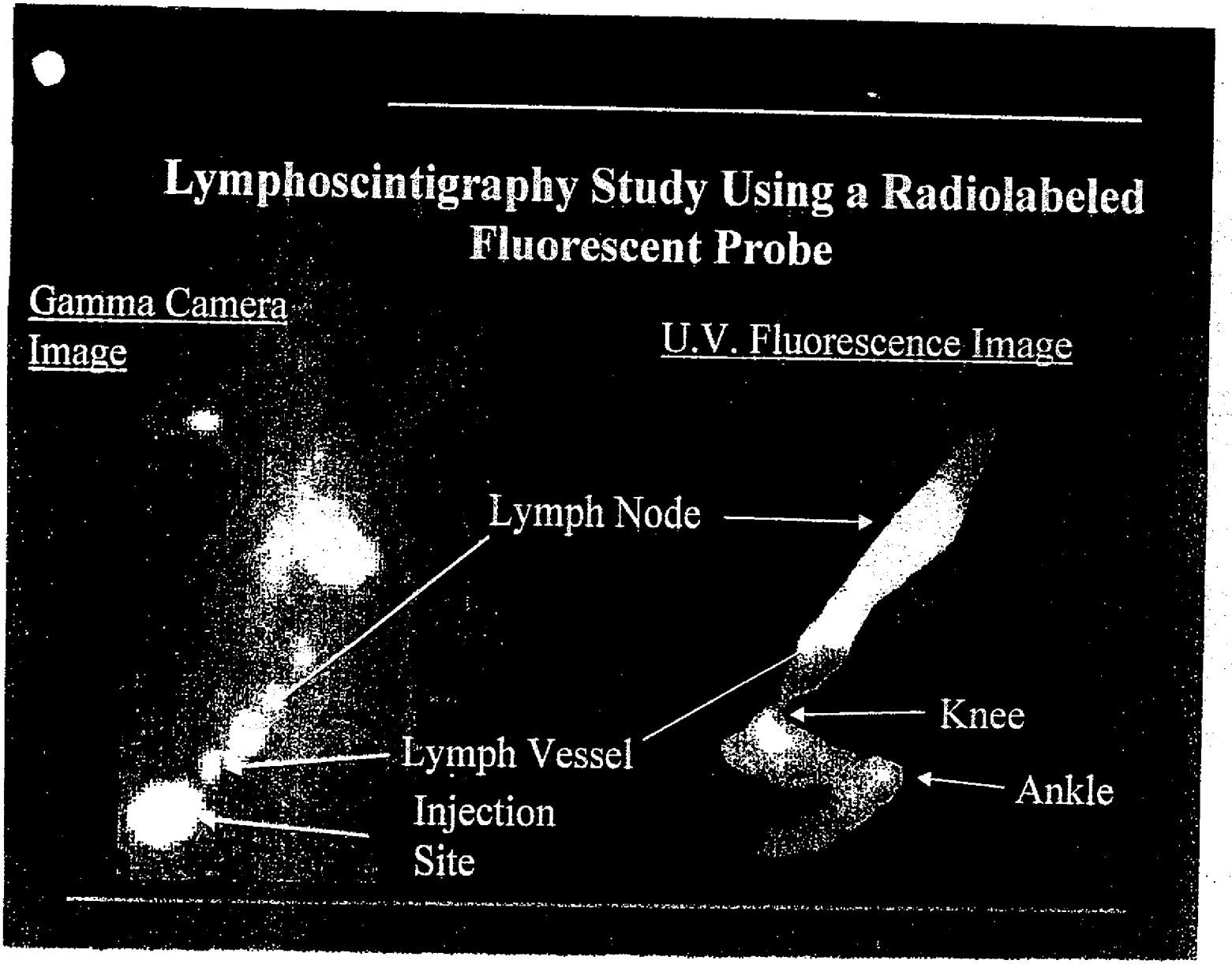
Lymphoscintigraphy Study Using a Radiolabeled Fluorescent Probe
Gamma Camera Image
U.V. Fluorescence Image
Lymph Node
Lymph Vessel
Injection Site
Knee
Ankle

Figure 4a

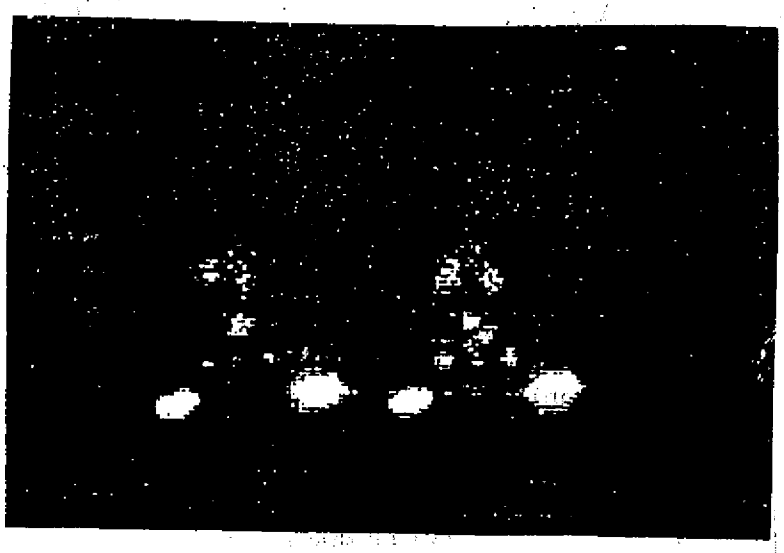

Figure 4b

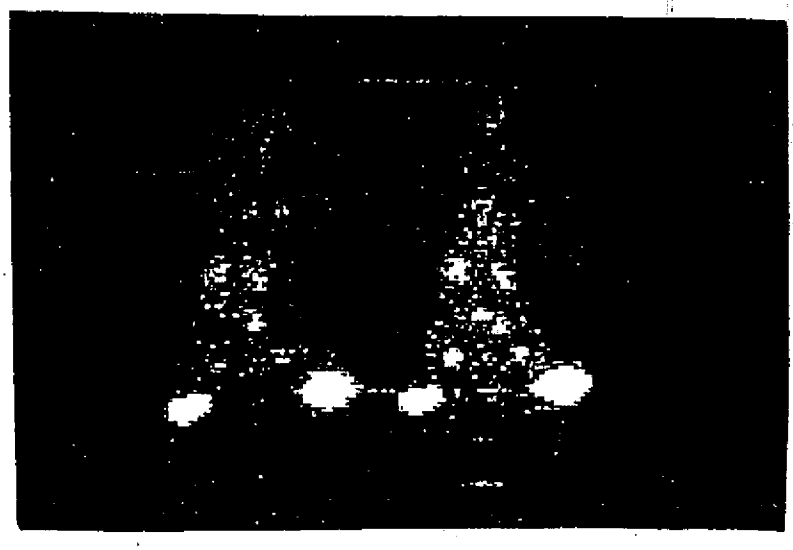

Figure 4c

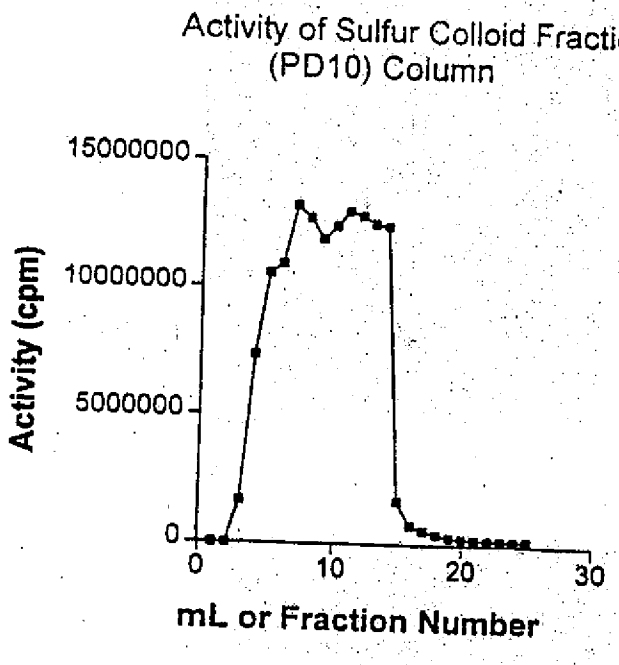

Figure 5a

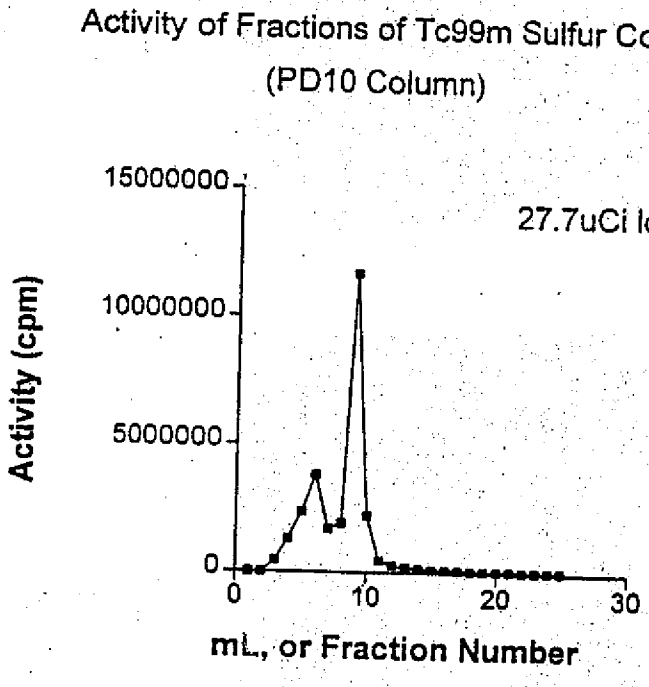

Figure 5b

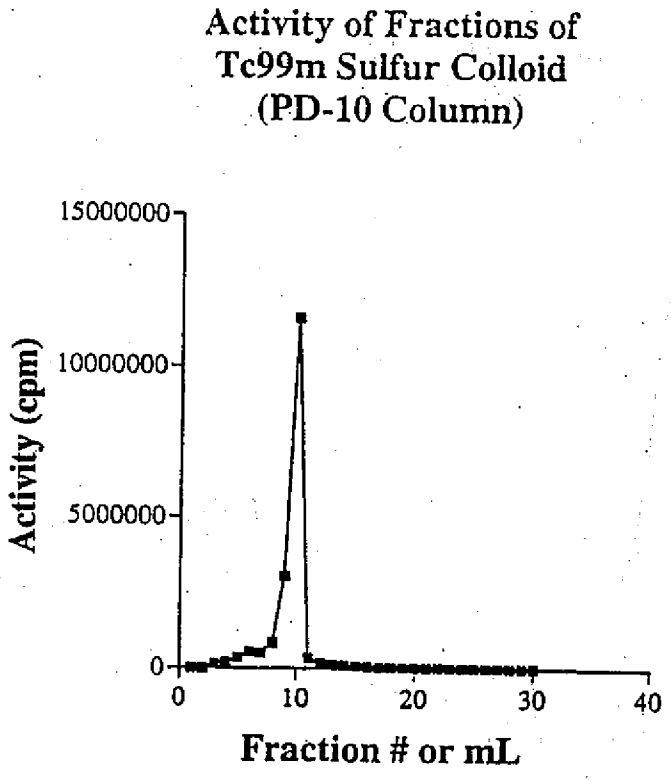

Figure 6a

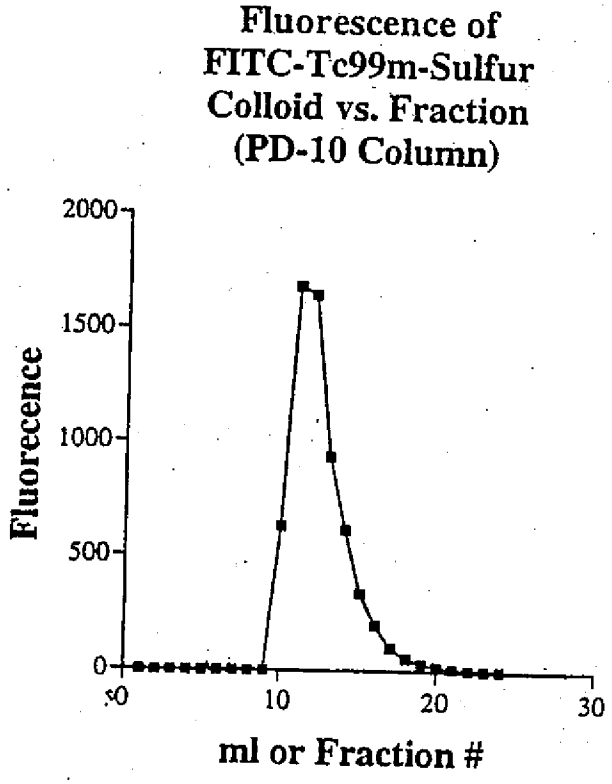

Figure 6b

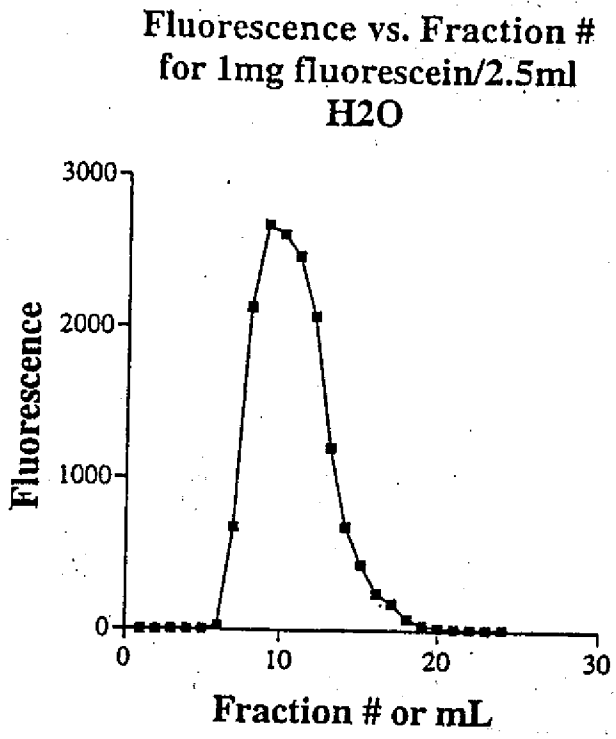

Figure 6c

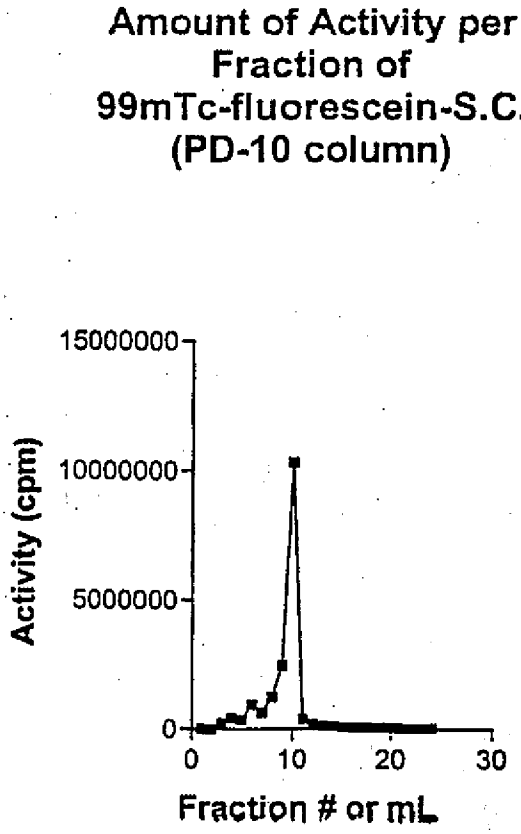

Figure 6d

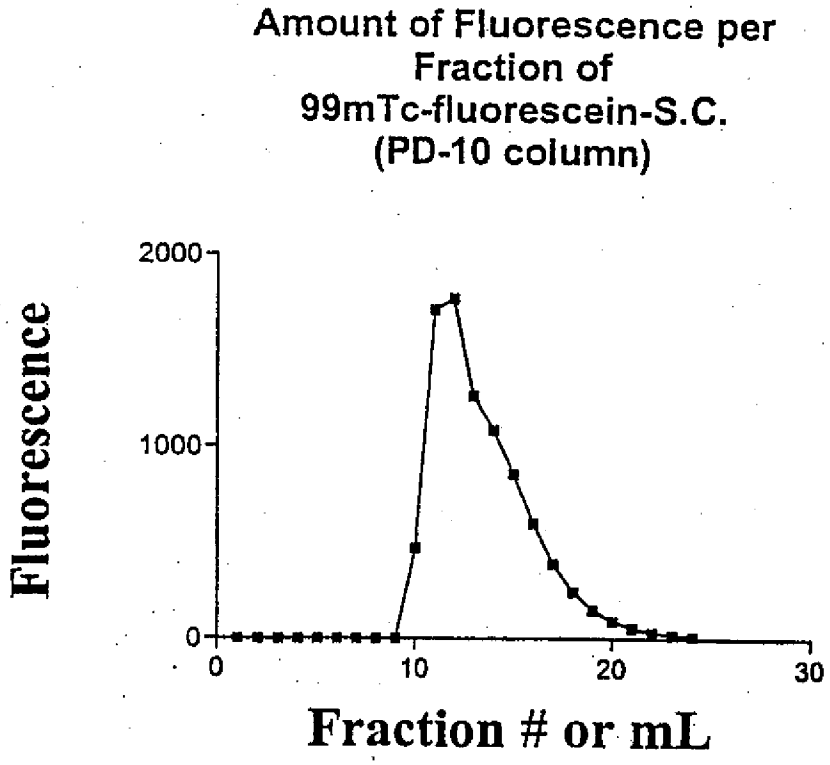

Figure 6e

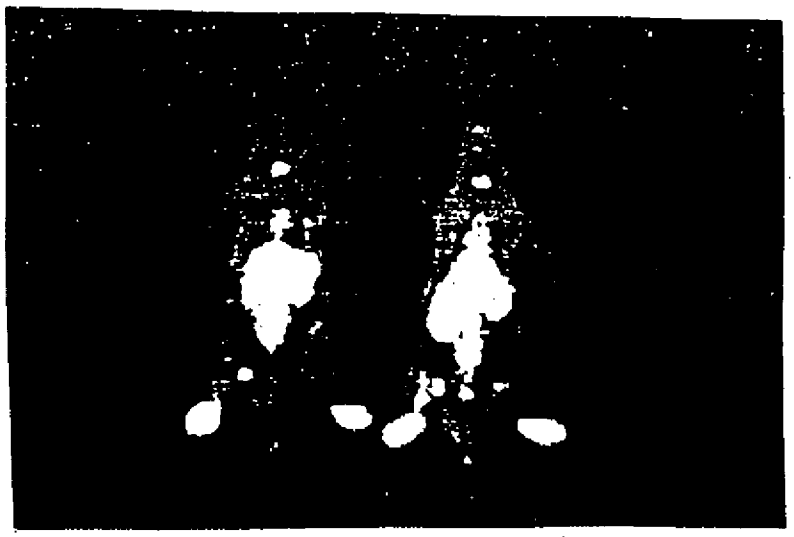

Figure 6f

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except US)*: **RESOLUTION PHARMACEUTICALS, INC.** [CA/CA]; 6850 Goreway Drive, Mississauga, Ontario L4V 1V7 (CA).

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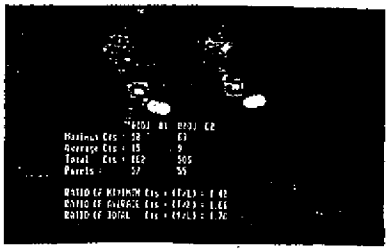
(74) Agent: **DONAHUE ERNST & YOUNG LLP**; Ernst & Young Tower, Toronto-Dominion Centre, 222 Bay Street, Suite 1800, P.O. Box 197, Toronto, Ontario M5K 1H6 (CA).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO,

[Continued on next page]

(54) Title: RADIOPHARMACEUTICALS AND METHODS FOR IMAGING



(57) Abstract: This invention discloses the concept of incorporating a radioactive agent and various dyes to enhance lymphatic drainage, sentinel and lymph nodes. The use of a gamma emitting radionuclide such as Tc-99m allows the localization of the lymph node(s) that allows the surgeon to initially plan the surgical procedure. On the day of the study the radiopharmaceutical may need to be injected imaged and with the skin marked externally to assist the surgeon in locating the node during surgery. In order to facilitate the surgical probe a gamma detecting surgical probe can assist in providing the relative location of the node. This procedure has been found to be useful, however it has often been difficult utilizing this procedure to find all of the nodes. This invention would incorporate the use of a radioactive probe with a dye, the addition of the dye into the particles would allow the physician to more rapidly indentify lymphatic channels, sentinel and other node(s) and allow them to be excised which would dramatically reduce the surgical time for the patient.

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NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Inte. onal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K49/00 A61K51/12 //A61K103:10,A61K123:00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, MEDLINE, CHEM ABS Data, CANCERLIT, DISSERTATION ABS, EMBASE, EPO-Internal WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HORIUCHI K ET AL: "LIGANDIN BINDING PHTHALEIN COMPLEXONE COMPLEX OF TECHNETIUM FOR HEPATIC FUNCTION STUDIES" EUROPEAN JOURNAL OF NUCLEAR MEDICINE, vol. 16, no. 3, 1990, pages 137-142, XP000990394 ISSN: 0340-6997 abstract figure 1 page 139, right-hand column, paragraph RESULTS -page 142, left-hand column --- -/--	1-11,14, 15, 31-33, 35-42

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2001	02/04/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Dullaart, A

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INTERNATIONAL SEARCH REPORT

Inte ional Application No
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Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Int. ..ional Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5 667 764 A (LIN CHIA-EN ET AL) 16 September 1997 (1997-09-16) examples ---	1-11,14, 15, 31-33, 35-42
X	TERASHIMA M ET AL: "ADP-ribosylation of tuftsin suppresses its receptor-binding capacity and phagocytosis-stimulating activity to murine peritoneal macrophages." FEBS LETTERS, JUL 21 1997, VOL. 412, NO. 1, PAGE(S) 227-32, XP000990355 abstract figure 1 page 228, right-hand column, paragraph 2.5 -page 229, left-hand column page 229, paragraph RESULTS AND DISCUSSION --- -/--	1-11,14, 15, 31-33, 35-42

INTERNATIONAL SEARCH REPORT

Inte ional Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	ORDA R ET AL: "POST SPLENECTOMY SPLENIC ACTIVITY" ANNALS OF SURGERY, vol. 194, no. 6, 1981, pages 771-774, XP000990400 ISSN: 0003-4932 abstract page 771, paragraph MATERIALS AND METHODS page 773, paragraph DISCUSSION ---	1-42
Y	SOM P ET AL: "Diagnosis of osteomyelitis and soft tissue infection using a Tc-99m labeled tuftsin-analog peptide." JOURNAL OF NUCLEAR MEDICINE, vol. 38, no. 5 SUPPL., 1997, page 132P XP000990357 44th Annual Meeting of the Society of Nuclear Medicine;San Antonio, Texas, USA; June 1-5, 1997 ISSN: 0161-5505 the whole document ---	1-42
Y	PEERS S H ET AL: "Imaging a model of colitis with RP128, a Tc-99m-chelated tuftsin antagonist." JOURNAL OF NUCLEAR MEDICINE, vol. 36, no. 5 SUPPL., 1995, page 114P XP002102963 42nd Annual Meeting of the Society of Nuclear Medicine;Minneapolis, Minnesota, USA; June 12-15, 1995 ISSN: 0161-5505 the whole document ---	1-42
Y	AMOSCATO AA ET AL: "Receptor-mediated internalization of tuftsin by human polymorphonuclear leukocytes." JOURNAL OF THE RETICULOENDOTHELIAL SOCIETY, JUL 1983, VOL. 34, NO. 1, PAGE(S) 53-67, XP000990391 abstract page 54, paragraph FLUORESCENT LABELING OF TUFTSIN page 56, paragraph BINDING OF FLUORESCEINATED TUFTSIN TO HUMAN PMN page 58, last paragraph -page 60 tables 1-4 page 63, paragraph REDISTRIBUTION OF ALHPA-FITC-TUFTSIN ON PERIPHERAL BLOOD MONOCYTES --- -/--	1-42

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/CA 00/00661

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	AMOSCATO AA ET AL: "Receptor-mediated internalization of tuftsin." ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, 1983, VOL. 419, PAGE(S) 114-34, XP000990445 page 115 page 117, paragraph RESULTS -page 120, paragraph BIOLOGICAL ACTIVITY OF FITC-TUFTSIN figures	1-42
Y	HUNG JOSEPH C ET AL: "Filtered technetium-99m-sulfur colloid evaluated for lymphoscintigraphy." JOURNAL OF NUCLEAR MEDICINE, vol. 36, no. 10, 1995, pages 1895-1901, XP002163258 ISSN: 0161-5505 cited in the application the whole document	1-42
Y	ESHIMA DENNIS ET AL: "Technetium-99m-sulfur colloid for lymphoscintigraphy: Effects of preparation parameters." JOURNAL OF NUCLEAR MEDICINE, vol. 37, no. 9, 1996, pages 1575-1578, XP002163259 ISSN: 0161-5505 cited in the application the whole document	1-42
Y	US 5 866 544 A (GOODBODY ANNE ET AL) 2 February 1999 (1999-02-02) examples	1-42
Y,P	WO 99 51628 A (DU PONT PHARM CO) 14 October 1999 (1999-10-14) page 26, line 33 -page 27, line 8 examples	1-42

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-42 in part

Present claims 1-42 relate to methods, compositions and kits characterised by an extremely large number of possible active agents. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the active agents claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. As a consequence, the initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claim(s) may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breadth of the claim(s) is impossible. Consequently, the search has been restricted to the active agents mentioned in the examples, and to the general idea of combining contrast agents for scintigraphy and for fluorescent imaging.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/CA 00/00661

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